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Reduced Bacterial Colonisation on Surfaces Coated with Silicone Nanostructures

*Margrith Meier, Valentin Dubois, Stefan Seeger**

Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich,
Switzerland

HIGHLIGHTS:

- Hydrophobic silicone nanostructures were coated on glass.
- Bacterial colonisation on the different silicone nanostructures was investigated.
- A decrease was observed on superhydrophobic coatings under static conditions.
- The reduction under dynamic conditions depended on the bacterial species.

KEYWORDS. Superhydrophobic, Bacterial adhesion, Nanostructures, Nanofilaments, Silicone

Abstract

Bacterial adhesion on silicone nano- and microstructures is investigated in stagnant and flow experiments. Static adhesion tests are performed in 0.9 % NaCl solution. These experiments reveal that the number of *Staphylococcus epidermidis* (*S. epidermidis*) and *Escherichia coli* (*E. coli*) adhering to glass surfaces can significantly be reduced if silicone nanofilament and rod coatings are present. Further, flow experiments are conducted in a parallel-plate flow chamber using 0.9 % NaCl solution and artificial urine as medium. Silicone nanofilament coated surfaces are compared

to uncoated glass surfaces. *E. coli* colonisation on filament coated surfaces is reduced for at least 24 h in 0.9 % NaCl solution, while in artificial urine no reduction is observed after 24 h. *S. epidermidis* shows converse adhesion behaviour. Here, initial adhesion on nanofilaments is promoted but the number of adherent *S. epidermidis* seems to decrease after extended contact time. The obtained results demonstrate that superhydrophobic silicone surfaces significantly reduce bacterial colonisation under stagnant and dynamic conditions. However, the bacterial adhesion behaviour depends on the architecture of the silicone nano- and microstructures and the bacterial species investigated.

1. Introduction

Biofilm formation and colonisation is a frequent consequence of bacterial surface adhesion. Although some biofilms are favourable such as for bioremediation in sewage treatment systems, the adhesion of bacteria is a critical issue in many fields of industrial and medical applications.[1–3] Examples for such undesired adhesion are bacterial colonisation on biomedical materials causing infections or biofilms in food processing facilities which lead to food spoilage.

To overcome these detrimental implications several chemical, physical and biological approaches for antimicrobial surface modifications have been suggested.[4–7] One strategy is based on biocidal active surfaces which are modified with antimicrobial substances such as polymers, antibiotics, enzymes or heavy metals like silver.[6] Bacteria repelling surfaces represent a second approach. They are realized, e.g., by polymer brushes or hydrogel coatings.[6–8] These anti-adhesive surfaces do not involve biocides which is an advantage given the increasing concerns about bacterial resistance.[9] In recent years, superhydrophobic micro- or nanotextured materials have also been investigated for their anti-adhesive properties. Some studies indeed reported

reduced adhesion of bacteria to superhydrophobic materials such as fluorinated silica, nanostructured silicone elastomers or titanium dioxide nanotube films modified with fluorinated silanes.[10–16] However, others found no reduction after prolonged exposure to bacteria contaminated medium.[17–20] A reason for the decreased bacterial adhesion on superhydrophobic surfaces might be the entrapped air layer - called plastron - in between the solid/liquid interface.[10] The plastron accounts for that only the highest surface protrusions of the micro- and nanostructured topography contact the medium.[21,22] Therefore, there is less surface contact area available for the bacteria compared to a completely wetted non-superhydrophobic surface.[10,12,19] The surface maintains its superhydrophobicity as long as the air layer is intact. Nonetheless, the air can dissolve into the surrounding liquid which increases the available surface contact area for bacterial binding. The plastron lifetime depends on several parameters like surface tension, temperature or hydrostatic pressure.[23]

Some years ago, we have shown that superhydrophobic surfaces can be made from silanes. Silanes are the precursor molecules for a polycondensation reaction leading to polysiloxanes, also well known as silicones.[24–27] Silicone material is biocompatible and therefore used in medicine and household to name a few application areas.[27,28] Many surfaces coated with polysiloxanes exhibit low surface free energies. Combining such surfaces with nano- or microscale roughness makes them superhydrophobic.[29–31] Nanostructured silicone surfaces are thus interesting candidates to be investigated for their bacterial antiadhesive properties. We are able to obtain surfaces with different nanostructures such as filaments, rods or volcanoes from the same precursor organosilane molecule.[32,33] Among them the filaments are the longest known and best investigated structures.[24,34,35] Beside the use as superhydrophobic coating, they have also been studied as high surface area supports for various purpose such as biocides and catalysts.[36–39]

In this study, we explore whether silicone coatings consisting of filaments, rods and volcanoes can hinder bacterial colonisation of *Escherichia coli* (*E. coli*) and *Staphylococcus epidermidis* (*S. epidermidis*). For these purposes, we perform static bacterial adhesion experiments in 0.9 % NaCl solution to elaborate how the different textures hamper bacterial attachment compared to pristine glass surfaces. Additionally, the bacterial adhesion on silicone nanofilaments is verified in a dynamic flow set-up using artificial urine as medium to simulate more practical conditions.

2. Experimental Section

2.1. Materials

Trichloroethylsilane (≥ 97 %, Merck) was stored and handled under nitrogen atmosphere. LB agar (Invitrogen), LB broth base (Invitrogen), peptone from casein and other animal proteins (Sigma-Aldrich), yeast extract (BioChemica), lactic acid solution (≥ 98 %, Sigma-Aldrich), citric acid (Sigma-Aldrich), sodium bicarbonate (≥ 99.7 %, Sigma-Aldrich), urea (> 99.5 %, Fluka), uric acid (≥ 99 %, Sigma-Aldrich), creatinine (≥ 98 %, Sigma-Aldrich), calcium chloride dihydrate (≥ 99 %, Sigma-Aldrich), sodium chloride (≥ 99.5 %, Sigma-Aldrich), iron(II) sulfate heptahydrate (≥ 99.0 %, Fluka), magnesium sulfate (≥ 98 %, Sigma-Aldrich), sodium sulfate (≥ 99 %, Sigma-Aldrich), potassium phosphate monobasic (≥ 99.0 %, Sigma-Aldrich), potassium phosphate dibasic (≥ 98 %, Sigma-Aldrich) and glutaraldehyde solution (25 %, Sigma-Aldrich) were used as received. Ultrapure water (18.2 M Ω cm) was prepared by a Simplicity water purification system (Millipore, USA).

2.2. Sample preparation

The different nano- and microstructures were obtained by following the procedure described elsewhere.[32,33] Microscope glass slides (Menzel Gläser, Germany) were ultrasonicated in a

10 % (v/v) aqueous solution of Deconex 11 Universal (Borer Chemie AG, Switzerland) for 15 min at 50 °C, rinsed with ultrapure water and dried under a stream of nitrogen. Cleaned glass slides were put upright in a desiccator (volume: 6.5 L). The sealed desiccator was then flushed with humidified nitrogen for 1 h to obtain a relative humidity of ≈ 40 % for filaments, ≈ 70 % for rods and ≈ 90 % for volcanoes inside the desiccator. Afterwards, trichloroethylsilane (500 μ l, 3.8 mmol) was injected through a septum and the gas phase reaction proceeded for at least 4 h. Coated glass slides were rinsed with bidistilled water, dried under a stream of nitrogen and annealed at 200 °C for 2 h.

2.3. Bacterial adhesion experiments

E. coli (DSM 498) and *S. epidermidis* (ATCC 14990) were grown overnight from a frozen stock in LB broth base at 37 °C, inoculated in fresh LB broth base and grown to $\approx 1 \times 10^8$ *E. coli* CFU mL⁻¹ or $\approx 6 \times 10^7$ *S. epidermidis* CFU mL⁻¹ determined by optical density at 600 nm using a UV/VIS/NIR spectrometer Lambda 900 (Perkin-Elmer, USA) and viable counts on LB agar plates.

Static adhesion experiments were conducted in a staining jar which was filled with 99 mL 0.9 % (w/w) NaCl aqueous solution and 1 mL bacterial culture. Then, filament, rod and volcano coated glass slides as well as an uncoated glass slide as reference were placed inside. The staining jar was incubated for 3 h at 37 °C. Special care was taken to avoid any vibrations. The substrates were removed and immersed slowly in 0.9 % (w/w) NaCl aqueous solution to rinse non-adherent bacteria. Afterwards, the substrates were transferred to a 50 mL conical tube (Greiner, Germany) filled with 0.9 % (w/w) NaCl aqueous solution and sonicated on ice for 10 min to remove adherent bacteria. Bacterial suspensions were serially diluted and plated on LB agar.

Dynamic adhesion experiments were performed in a parallel-plate flow chamber (GlycoTech, USA) with dimensions of 5.8 cm x 1.0 cm x 0.025 cm (L x W x H). Two parallel-plate flow chambers with either a filament coated or an uncoated glass slide were run simultaneously. 1 mL bacterial culture was suspended in 99 mL 0.9 % (w/w) NaCl aqueous solution or artificial urine (pH 7.3) prepared according to a procedure reported by Brooks and Keevil.[40] The bacterial suspension was heated to 37 °C and circulated by means of a peristaltic pump (Ismatec, Switzerland) through the flow chambers for 3 h or 24 h at a flow rate of 97 $\mu\text{L min}^{-1}$ which yields a shear rate of 15 s^{-1} . [41] Then, 0.9 % (w/w) NaCl aqueous solution was circulated for another 30 min at the same flow rate to rinse non-adherent bacteria from the substrates. The substrates were transferred into a 50 mL conical tube (Greiner, Germany) filled with 0.9 % (w/w) NaCl aqueous solution and sonicated on ice for 10 min to remove adherent bacteria. Bacterial suspensions were serially diluted and plated on LB agar.

The number of adherent bacteria was given in CFU mm^{-2} and represented mean \pm standard error of the mean of three independent experiments. Relative bacteria adhesion was calculated using the formula: relative bacteria adhesion = (A/B) x 100 % with A = CFU mm^{-2} of adherent bacteria on coated glass slides and B = CFU mm^{-2} of adherent bacteria on uncoated controls. Data were analyzed by one-way analysis of variance (ANOVA) with Tukey–Kramer multiple comparisons test. Results were considered significant at $P < 0.05$.

2.4. Characterisation

A 10 μl amount of ultrapure water, 0.9 % (w/w) NaCl aqueous solution or artificial urine was used to determine the static contact angle on a contact angle system OCA (Dataphysics, Germany). The contact angle of the drop was analyzed at five different positions on each sample using SCA software (Dataphysics, Germany). The sliding angles were measured on a custom made tilting

device. For estimating bacteria wettability three different bacterial lawns were prepared for each species. The bacterial lawn was prepared by collecting bacterial cells on polycarbonate membranes (Whatman Nuclepore Track-Etched membranes) with pore size of 0.1 μm . Membranes containing a bacterial lawn were placed on a glass slides with double sided adhesive tape and allowed to air dry for 30 – 60 min before performing contact angle measurements. All contact angle and sliding angle measurements were done within 2 min after depositing the droplet.

The scanning electron microscopy (SEM) samples were sputtered with 15 nm platinum. The SEM images were recorded on a Zeiss Supra 50 VP (Zeiss, Germany) at 2 kV using the inlens detector. Samples which were investigated after having circulated bacteria inoculated 0.9 % (w/w) NaCl aqueous solution or artificial urine (pH 7.3) for 24 h at a flow rate of 97 $\mu\text{L min}^{-1}$ were fixed in a 3 % (v/v) glutaraldehyde solution buffered with 0.1 M phosphate buffer (pH 7.2) for 2 h at room temperature and subsequently dehydrated in a graded series of ethanol in water (35 %, 50 %, 70 %, 80 %, 90 %, 100 % v/v for 15 min each) prior to sputtering.

3. Results and Discussion

3.1. Sample Preparation

Glass slides were chosen as substrate since they have the appropriate size for the experimental set-up and thus facilitate the microbiological analysis. We achieved the synthesis of different morphologies by adjusting the relative humidity inside the CVD chamber according to previously published data.[32,33,35] Figure 1 depicts SEM images of the three silicone nano- and microstructures chosen for this study. These structures and their mechanism of formation were recently described in detail. [33] The relative humidity was adjusted to around 40 % to obtain filaments (cf. Figure 1a,d), while the formation of rods (cf. Figure 1b,e) required approximately

70 % relative humidity. The largest structures among them are volcanoes (cf. Figure 1c,f) and have been formed at around 90 % relative humidity.

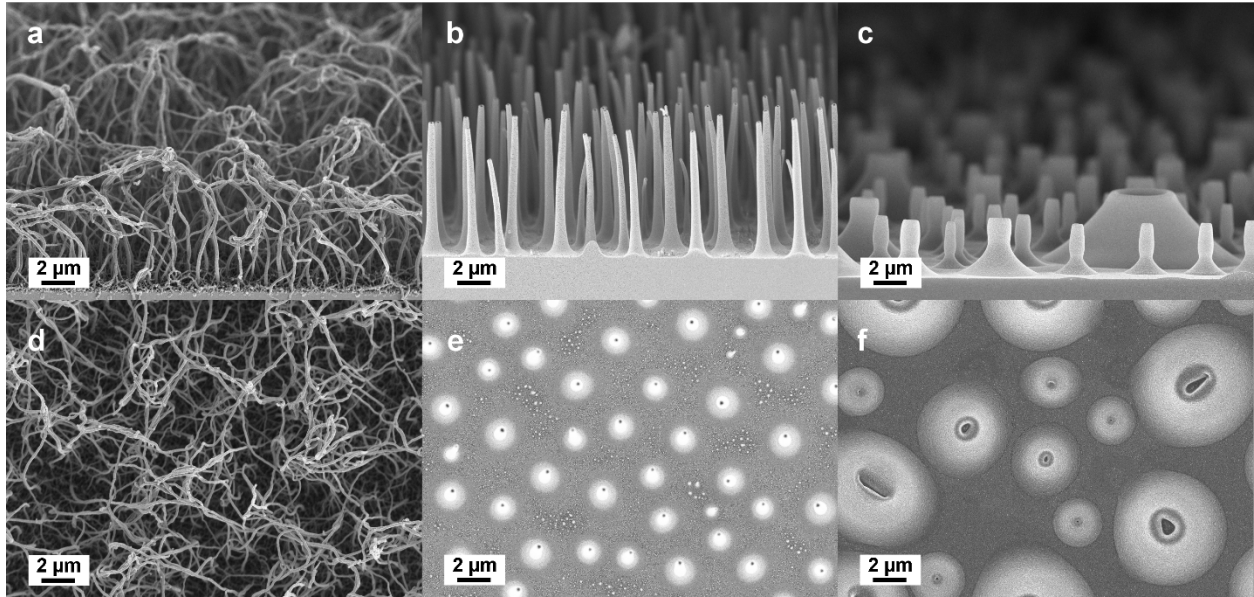


Figure 1. SEM images of nano- and microstructures obtained by varying relative humidity in the gas phase reaction: a,d) filaments; b,e) rods; c,f) volcanoes; a-c) side view and d-f) top view.

Static water contact angles and sliding angles were measured to investigate the wettability characteristics of the three structures prior to bacterial attachment testing. The medium used for bacterial adhesion experiments consisted of 0.9 % NaCl solution and artificial urine, respectively. As solutes affect the surface tension of water and consequently might influence the contact angle, we performed the contact angle measurements using 0.9 % NaCl solution and artificial urine as liquid for the drop.[30,42] The obtained results summarized in Table 1 were in line with previous reports.[32,35] They showed that glass slides coated with filaments or rods formed superhydrophobic surfaces with contact angles above 150° and sliding angles around 10° . Whereas surfaces coated with volcanoes were hydrophobic exhibiting a contact angle around 110° . By tilting the volcano coated glass slide the drop did not roll off but stuck to the surface. We assume

that the reduced roughness on the nanoscale of volcano modified surfaces accounts for the increased wettability.[29] Since we employed the same precursor and only varied the relative humidity to form the different structures, there should be no notable variation in surface chemistry.[33] In contrast to coated samples, a smooth and hydrophilic surface of a cleaned glass slide exhibited a contact angle below 10°.

Table 1. Static Contact Angle Measurements

	contact angle			sliding angle		
	filaments	rods	volcanoes	filaments	rods	volcanoes
water	165° ± 3°	174° ± 4°	117° ± 9°	10° ± 2°	5° ± 2°	-
0.9 % NaCl solution	164° ± 5°	168° ± 4°	111° ± 8°	11° ± 2°	6° ± 1°	-
artificial urine	159° ± 1°	167° ± 2°	110° ± 4°	16° ± 1°	8° ± 1°	-

- Drop does not roll off

3.2. Static Bacterial Adhesion Experiments

We performed static bacterial adhesion tests in 0.9 % NaCl solution at 37 °C to get an overview whether one of the three silicone coatings impede bacterial attachment. The microorganisms chosen for this study were the well-studied model organism *E. coli* and *S. epidermidis*, a bacterium associated to the human skin microbiome.[43,44] Although *S. epidermidis* is considered to be harmless, it can cause prosthetic device infections under certain circumstances.[45] The selected bacteria species also differ in cell shape and cell wall structures. *E. coli* is a rod-shaped Gram-negative bacterium while *S. epidermidis* is spherical and Gram-positive.

After immersing the coated glass slides and an uncoated control sample for 3 h inside bacteria inoculated 0.9 % NaCl solution, enumeration of viable bacteria attached to the substrates was determined by plate count. The diagrams in Figure 2 depict the colony forming units (CFU) per mm² of substrate. The samples modified with filaments or rods significantly reduced bacterial

adhesion compared to uncoated glass. Relative *E. coli* adhesion normalized to uncoated glass was $16 \% \pm 5 \%$ for filaments and $21 \% \pm 7 \%$ for rods. By contrast, volcanoes did not decrease *E. coli* attachment. They exhibited a relative adhesion of $95 \% \pm 15 \%$. Static *S. epidermidis* adhesion experiments revealed a similar trend except that in absolute numbers less bacteria adhered to the surfaces. The relative *S. epidermidis* adhesion amounted to $5 \% \pm 3 \%$ for filaments, $12 \% \pm 7 \%$ for rods and $87 \% \pm 19 \%$ for volcanoes. These results clearly indicate that filament and rod coated surfaces reduce bacterial adhesion under static conditions. However, it should be taken into consideration that in static adhesion experiments the forces which occur by removing or rinsing the samples might be strong enough to detach loosely adherent bacteria.[46]

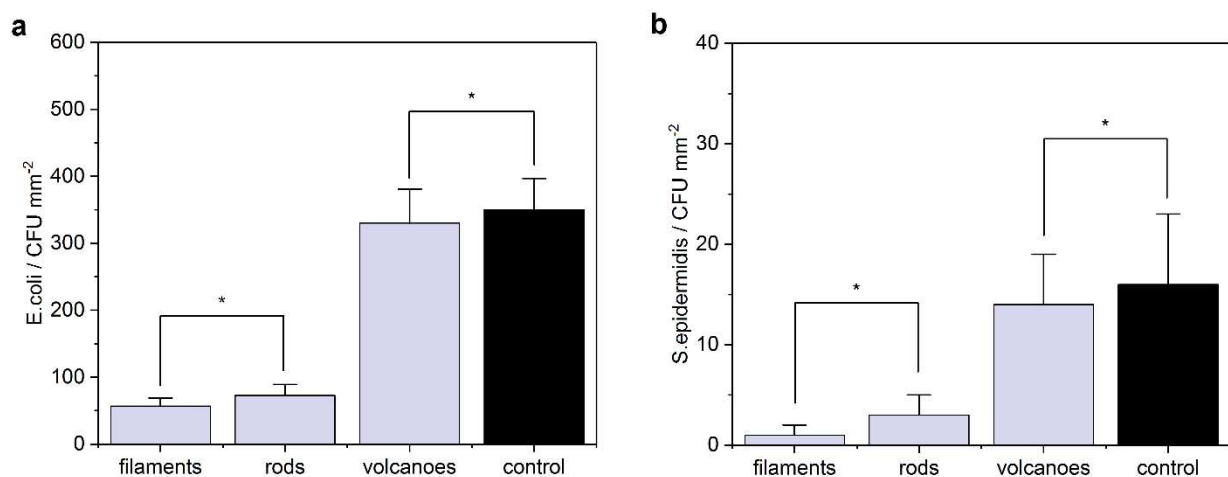


Figure 2. Results of static adhesion experiments in 0.9 % NaCl solution performed over a 3 h time period. a) Number of *E. coli* colony forming units (CFU) per mm² of sample. b) Number of *S. epidermidis* CFU per mm² of sample. Uncoated glass slides served as control. Asterisk denotes not significant differences.

3.3. Dynamic Bacterial Adhesion Experiments

In many applications, surfaces are exposed to moving liquid phases. Therefore, bacterial adhesion was studied in a parallel-plate flow chamber. This dynamic experimental set-up allows

to investigate bacterial adhesion under those circumstances. Additionally, it permits to control hydrodynamic conditions.[47] We investigated the dynamic adhesion on filament coated glass slides and uncoated controls. Rod and volcano coated surfaces were omitted in the dynamic adhesion experiments because the findings obtained from the static adhesion tests indicated that the number of adherent bacteria per mm^2 was similar for filaments and rods and for volcanoes and control (cf. Figure 2). A low fluid flow induces a slower bacterial colonisation due to a smaller mass transport, while a too high flow could stimulate detachment or even prevent adhesion of microorganisms.[48,49] We therefore used a similar shear rate as found in urinary catheters.[50] The flow rate was adjusted to $97 \mu\text{L min}^{-1}$ which yields a shear rate of 15 s^{-1} for the flow chamber applied in this study. As the availability of nutrients influences bacterial adhesion to surfaces, we have also included nutrient-rich artificial urine as medium for the bacterial suspension.[51] Plate count enumerations at defined time periods revealed that the number of *S. epidermidis* remained roughly constant in 0.9 % NaCl solution. By contrast, the same experiments carried out in artificial urine resulted in bacterial growth.

The bacterial suspensions were circulated for 3 h and 24 h respectively. Diagrams in Figure 3 show the number of adherent CFU per mm^2 . *E. coli* generally adhered to the samples in a higher number than *S. epidermidis*. This finding conformed to the static adhesion experiments. Filaments reduced *E. coli* adhesion in 0.9 % NaCl solution (cf. Figure 3a,c). Relative adhesion normalized to control was $22 \% \pm 16 \%$ after 3 h and $46 \% \pm 24 \%$ after 24 h. In contrast to these results, *S. epidermidis* showed enhanced bacterial colonisation on filaments in 0.9 % NaCl solution (cf. Figure 3b,d). Relative adhesion after 3 h was $748 \% \pm 76 \%$ and decreased after 24 h to $244 \% \pm 73 \%$. Notably, using artificial urine as medium revealed after 3 h a relative *S. epidermidis* adhesion of $263 \% \pm 72 \%$ which declined after 24 h to $36 \% \pm 21 \%$. This outcome

indicates a reduced *S. epidermidis* attachment compared to the control after an extended time period. However, *E. coli* demonstrated an opposite adhesion behaviour on filaments in artificial urine. They had a reduced attachment after 3 h and the respective relative adhesion was $19 \% \pm 18 \%$. Nonetheless, the initial reduction disappeared after extending the flow time to 24 h. The relative *E. coli* adhesion increased to $139 \% \pm 18 \%$. Representative SEM images of filament coated samples which have been exposed for 24 h to bacteria inoculated 0.9 % NaCl solution or artificial urine are shown in Figure 4. It should be mentioned that the illustrated structure of the filament coated surfaces might have been influenced by the fixation and dehydration procedure during SEM sample preparation. Nevertheless, these images confirmed the general observation that *E. coli* adhered in a greater number than *S. epidermidis* to the samples. Overall, the dynamic flow experiments revealed that superhydrophobic surfaces retard bacterial adhesion relative to uncoated glass slides. However, the impact depends on the bacterial species. Although the coating reduces adhesion for *E. coli*, it seems to promote initial adhesion for *S. epidermidis*. Further research including different bacterial species will be necessary to provide a complete picture of this species selectivity of silicone nanofilament coated surfaces.

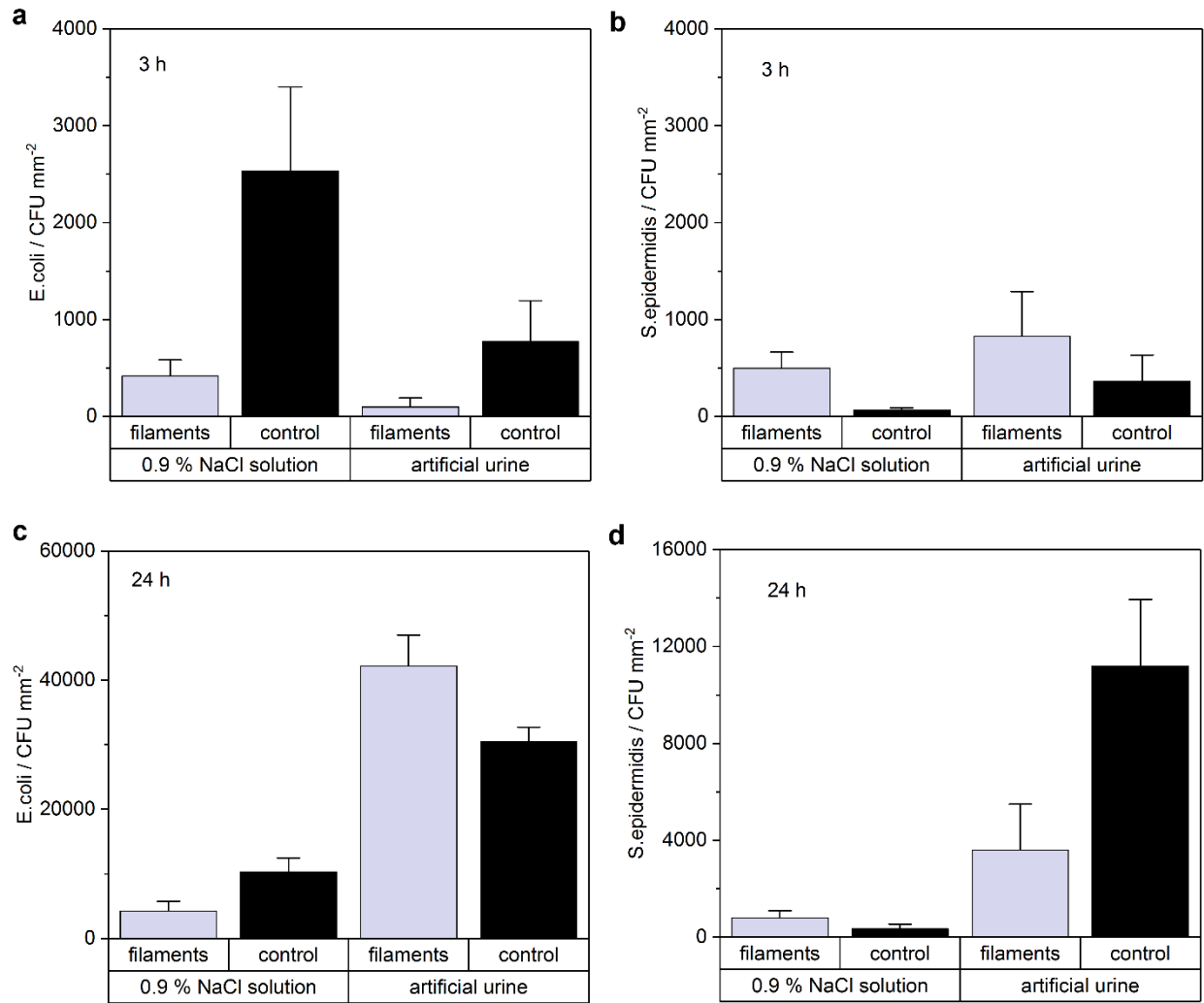


Figure 3. Results of dynamic adhesion experiments in 0.9 % NaCl solution and artificial urine. a) Number of adherent *E. coli* after 3 h of flow. b) Number of adherent *S. epidermidis* after 3 h of flow. c) Number of adherent *E. coli* after 24 h of flow. d) Number of adherent *S. epidermidis* after 24 h of flow.

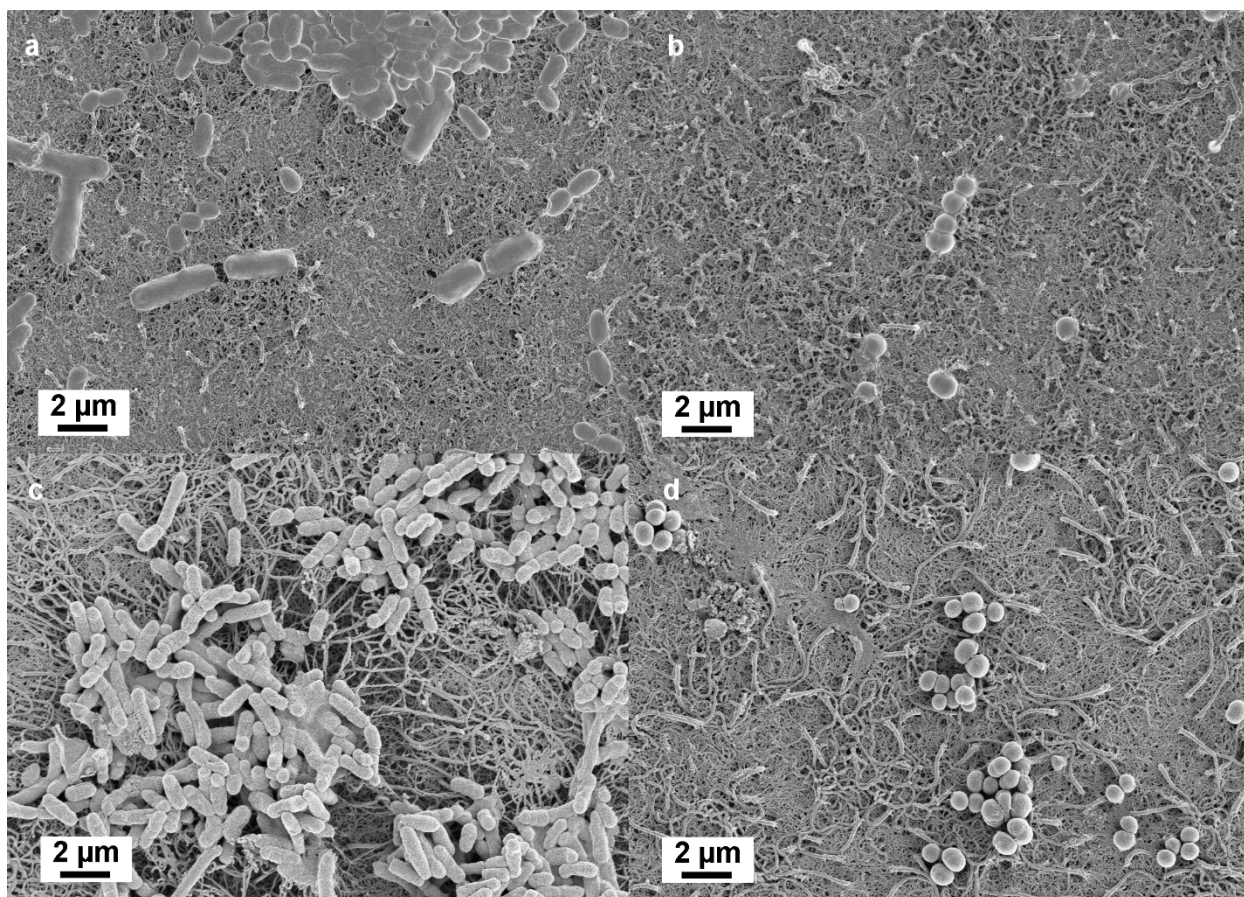


Figure 4. SEM images of filament coated samples which were exposed for 24 h to a) *E. coli* in 0.9 % NaCl solution, b) *S. epidermidis* in 0.9 % NaCl solution, c) *E. coli* in artificial urine and d) *S. epidermidis* in artificial urine.

3.4. Surface Analysis

Hydrodynamic parameters might affect the plastron lifetime and thus the surface hydrophobicity of the coated samples.[23] However, bacterial adhesion also depends on many other factors such as the composition of the bacteria surrounding medium, the surface properties of the bacteria and the solid substrate.[52–55] Artificial urine, for instance, contains beside different kind of salts and other molecules also peptides. These substances can adsorb or sediment on surfaces which might change the surface wettability of the samples over time and consequently influence bacterial adhesion.[56,57] Further investigations were therefore carried out to gather more detailed

information about the surface changes after flow experiments. The SEM image in Figure 5a depicts a filament coated surface which was exposed to artificial urine for 24 h. We compared this surface to SEM images of filament coatings which were exposed to 0.9 % NaCl solution (cf. Figure 5b) or water (cf. Figure 5c). The images only revealed some visible accumulations when the filaments were in contact with artificial urine. EDX spectra were acquired to examine the elemental surface composition of filament coated samples after exposure for 24 h to artificial urine (cf. Figure 5d) and 0.9 % NaCl solution (cf. Figure 5e). For comparison, EDX spectra of uncoated and non-exposed filament coated glass slides are shown in Figure 5f. The filament coated sample subjected to artificial urine caused a small nitrogen signal which was not detected for non-exposed and uncoated samples. This observation confirmed that some nitrogen containing components like urea, creatinine, ammonium or amino acids accumulated on the surface after cycling artificial urine for 24 h. Although artificial urine also contains sodium, magnesium, calcium and potassium salts, these signals could not be unambiguously attributed to the accumulations caused by the artificial urine. This is because the same signals also arise from the non-exposed and uncoated glass samples. The same reasoning applies for the carbon peak that might likewise be caused by the building blocks of the silicone nanofilaments which contain ethyl groups. Although SEM images of samples exposed to 0.9 % NaCl solution did not show visible accumulations, EDX analysis revealed a signal for sodium and chloride. In this respect, it is noteworthy that the sodium signal could also be caused by the glass substrate, but the chloride peak was only found for samples exposed to NaCl solution.

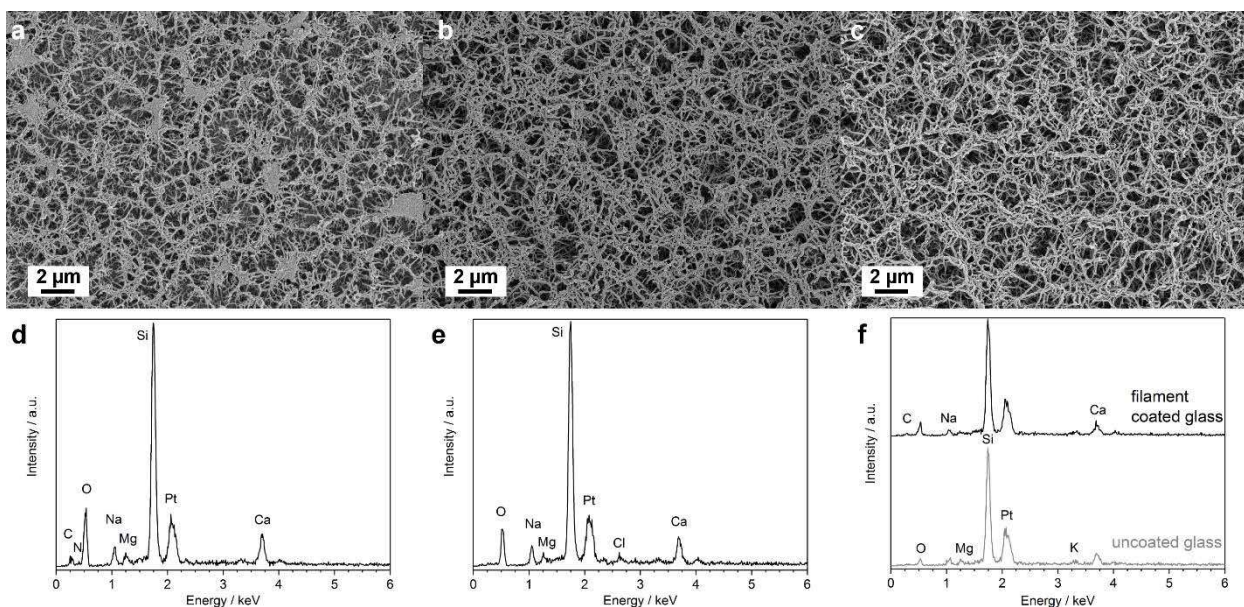


Figure 5. SEM images of filament coated samples which were exposed for 24 h to a) artificial urine, b) 0.9 % NaCl solution and c) water in a parallel-plate flow chamber. EDX spectra of filament coated samples which were exposed for 24 h to d) artificial urine and e) 0.9 % NaCl solution in a parallel-plate flow chamber. f) EDX spectra of an uncoated glass slide (grey) and a filament coated glass slide (black).

Wettability characteristics of the filament coating exposed to artificial urine and 0.9 % NaCl solution were investigated in closer detail to see how the surface wettability changed over time under the conditions used in the dynamic adhesion experiments. Diagrams in Figure 6 depict the determined water contact and sliding angles. The contact angle measured on samples exposed to 0.9 % NaCl solution only slightly decreased from $165^{\circ} \pm 3^{\circ}$ to $162^{\circ} \pm 3^{\circ}$ over a period of 24 h (cf. Figure 6a). However, the sliding angle clearly increased from $10^{\circ} \pm 2^{\circ}$ to $58^{\circ} \pm 7^{\circ}$ during this period (cf. Figure 6b). This finding corroborated the results obtained by EDX analysis and suggested that some salt molecules remained on the filament coated surfaces after circulating 0.9 % NaCl solution for 24 h. In contrast to the NaCl solution, the measured contact angle considerably diminished after exposure to artificial urine. It was found to be $110^{\circ} \pm 6^{\circ}$ after 24 h

(cf. Figure 6a). Additionally, we could not measure the sliding angles of filament coated samples subjected to artificial urine as the water drops did not roll off after tilting the surface. All in all, the obtained wettability characteristics were in line with the SEM and EDX observations.

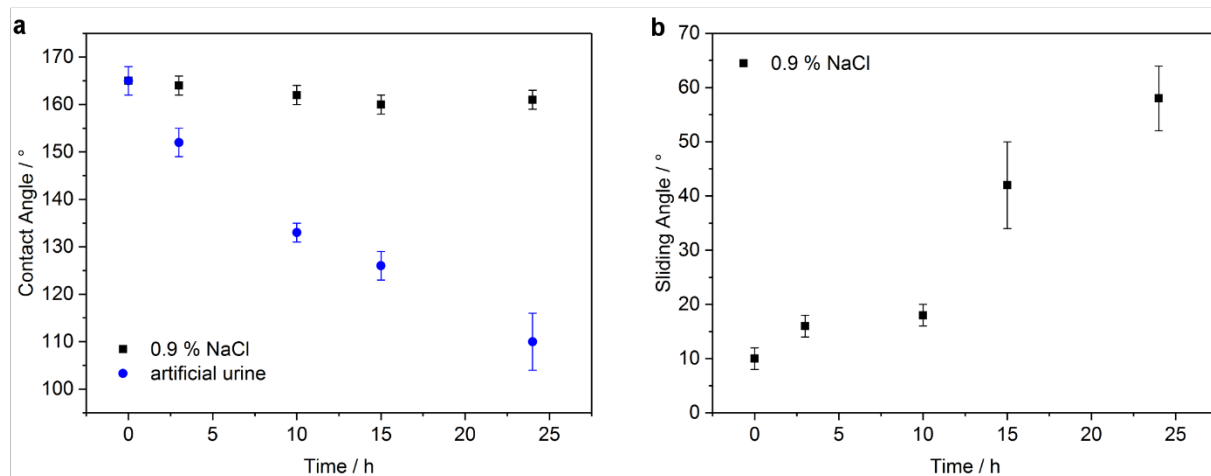


Figure 6. a) Water contact angles measured on filament coated samples which were exposed to 0.9 % NaCl solution or artificial urine at a shear rate of 15 s^{-1} for specified periods. b) Sliding angles measured on filament coated samples after specified times. 0.9 % NaCl solution at a shear rate of 15 s^{-1} was used as medium. Sliding angles were not determined on samples which were exposed to artificial urine since the water drop adhered to the surface.

As both bacteria species used in this study showed converse adhesion behaviour in the dynamic flow experiments, we tried to find out whether the surface hydrophobicity of the species could be relevant. Thus, we performed water contact angle measurements of bacterial lawns prepared on membrane filters to roughly estimate the difference in bacteria surface hydrophobicity. The bacteria contact angles effectively differed noticeably from each other. The contact angle determined for *E. coli* was $17^\circ \pm 2^\circ$ while $41^\circ \pm 3^\circ$ was obtained for *S. epidermidis*. These values suggest that the *E. coli* strain is more hydrophilic than the *S. epidermidis* strain used in this study.[54] However, apart from the wettability also other bacteria surface characteristics might

contribute to bacterial adhesion such as cell appendages or polysaccharides which may influence surface attachment by affecting electrostatic, van der Waals and hydrophobic interactions.[58] *S. epidermidis* and *E. coli* are both able to express capsular polysaccharides but *E. coli* additionally possesses flagella an organelle that provides mobility and may help to overcome electrostatic repulsion between bacterium and surface.[59,60]

4. Conclusion

In this study glass slides were coated with silicone nano- or microstructures and the adhesion of *E. coli* and *S. epidermidis* was evaluated in static as well as dynamic adhesion experiments. Bacterial adhesion to silicone coatings was compared to uncoated glass. In general, silicone nano- and microstructures can clearly reduce bacterial adhesion. However, it depends on several conditions, in particular the structure of the silicone architectures and the bacterial species. Coatings with filaments or rods successfully reduced the number of adherent bacteria in static adhesion experiments. By contrast, surfaces modified with volcanoes did not reduce the number of adherent bacteria. Changing from stagnant to flow conditions revealed that filaments retarded *E. coli* colonisation in 0.9 % NaCl solution for at least 24 h. However, the filament coating in artificial urine had about the same number of adherent bacteria after 24 h than uncoated glass surfaces. Remarkably, *S. epidermidis* showed a converse adhesion behaviour under dynamic experimental conditions. The initial adhesion was promoted but the number of adherent bacteria seemed to equalise or even decrease after 24 h. The obtained results indicated that silicone nanostructures could reduce bacterial colonisation. Further, the chemistry of silicone structures leaves plenty of room for chemical modifications and therefore improved anti-adhesion properties. Finally, our findings pointed out the importance of comparing different species and experimental conditions when evaluating bacterial adhesion on new coatings.

Corresponding Author

* E-mail: sseeger@chem.uzh.ch. Tel: +41 44 635 44 51.

Notes

The authors declare no competing financial interest.

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